AAMRL-TR-85-071
NMRI-85-56
AD# A163162

A ONE-YEAR INHALATION TOXICITY STUDY OF OTTO FUEL II

C. L. GAWORSKI
H. F. LEAHY
W. J. BASHE
J. D. MACEWEN
E. H. VERNOT
C. C. HAUN

UNIVERSITY OF CALIFORNIA, IRVINE
P.O. BOX 31009, OVERLOOK BRANCH
DAYTON, OHIO 45431-0009

R. H. BRUNER, LT COL, USA, VC
J. F. WYMAN, LCDR, USN, MSC

NAVAL MEDICAL RESEARCH INSTITUTE
TOXICOLOGY DETACHMENT
WRIGHT-PATTERSON AIR FORCE BASE, OHIO 45433

DECEMBER 1985

Approved for public release; distribution unlimited.

HARRY G. ARMSTRONG AEROSPACE MEDICAL RESEARCH LABORATORY
AEROSPACE MEDICAL DIVISION
AIR FORCE SYSTEMS COMMAND
WRIGHT-PATTERSON AIR FORCE BASE, OHIO 45433

NOTICES

When US Government drawings, specifications, or other data are used for any purpose other than a definitely related Government procurement operation, the Government thereby incurs no responsibility nor any obligation whatsoever, and the fact that the Government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data, is not to be regarded by implication or otherwise, as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use, or sell any patented invention that may in any way be related thereto.

Please do not request copies of this report from Air Force Aerospace Medical Research Laboratory. Additional copies may be purchased from:

National Technical Information Service
5285 Port Royal Road
Springfield, Virginia 22161

Federal Government agencies and their contractors registered with Defense Technical Information Center should direct requests for copies of this report to:

Defense Technical Information Center
Cameron Station
Alexandria, Virginia 22314

TECHNICAL REVIEW AND APPROVAL

AAMRL-TR-85-071

The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

BRUCE O. STUART, PhD
Director Toxic Hazards Division
Air Force Aerospace Medical Research Laboratory

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

1a. REPORT SECURITY CLASSIFICATION	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT: Available for Public Release; Distribution Unlimited
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S): AAMRL-TR-85-071 NMRI 85-56	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION: University of California 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION: AAMRL, Toxic Hazards Division
6c. ADDRESS (City, State and ZIP Code): Overlook Branch, P. O. Box 31009 Dayton, OH 45431	7b. ADDRESS (City, State and ZIP Code): AMD, AFSC Wright-Patterson AFB, OH 45433
8a. NAME OF FUNDING/SPONSORING ORGANIZATION 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: F33615-80-C-0512
8c. ADDRESS (City, State and ZIP Code)	10. SOURCE OF FUNDING NOS.

PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT NO.
622027	6302	NAVY	

11. TITLE (Include Security Classification): A One-Year Inhalation Toxicity Study of Otto Fuel II

12. PERSONAL AUTHOR(S): C.L. Gaworski, J.D. MacEwen, E.H. Vernot, C.C. Haun, H.F. Leahy, W.J. Bashe,

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Yr., Mo., Day)	15. PAGE COUNT
Technical Report	FROM 7/1980 TO 7/1985	DECEMBER 1985	31

16. SUPPLEMENTARY NOTATION: Naval Medical Research Institute Report No. 85-56

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)		
FIELD	GROUP	SUB. GR.	PGDN	Torpedo Fuel	1-2 Propylene glycol dinitrate
			Rat	Inhalation	Methemoglobin
			Dog	Mouse	Otto Fuel II

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

A 1-year industrial type (6 hours/day, 5 day/week) inhalation exposure was conducted to evaluate the oncogenic potential of the torpedo fuel, Otto Fuel II. Exposure levels of 1.4 mg/m³ and 240 mg/m³ were based on analyzed 1-2 propylene glycol dinitrate (PGDN) concentrations, the major component. Dogs (male and female beagle), rats (male and female Fischer 344), and mice (male and female C57BL/6) were exposed to the lower concentration, while only rats and mice were exposed to the higher concentration. Dogs appeared to be the most sensitive species tested with measurable reductions in RBC, hematocrit, and hemoglobin levels. Mildly increased methemoglobin was also observed in dogs exposed to 1.4 mg/m³ and rats exposed to 240 mg/m³. No other significant effects were noted in rats exposed at either concentration. Microscopic examination of tissues collected from dogs, rats, and mice exposed to Otto Fuel II failed to suggest any significant exposure related non-neoplastic changes. Similarly, exposure to Otto Fuel II also failed to increase the incidence of neoplastic changes. Rare primary bone tumors were noted in four rats

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT. ☐ DTIC USERS ☐	

22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE NUMBER (Include Area Code)	22c. OFFICE SYMBOL
M. K. Pinkerton	(513) 255-3364	AAMRL/THT

DD FORM 1473, 83 APR EDITION OF 1 JAN 73 IS OBSOLETE.

SECURITY CLASSIFICATION OF THIS PAGE

12. R. H. Bruner, J. F. Wyman

19. exposed to PGDN. However, the incidence of these tumors in exposed rats was not increased in a dose related fashion despite the extreme difference in the PGDN concentrations tested.

PREFACE

This document constitutes the final report on a One-Year Inhalation Toxicity Study of Otto Fuel II. The research covered a period from July 1977 through July 1983 and was performed under Contract Nos. F33615-80-C-0512 and F33615-76-C-5005. M. K. Pinkerton and K. C. Back, Ph.D. served as technical contract monitors for the Air Force Aerospace Medical Research Laboratory.

This work was sponsored by the U. S. Navy under the direction of LCDR M. J. Cowan, Jr., MSC, USN, Ret.; and Capt. D. E. Uddin, MSC, USN; and identified as Work Unit Number MF 58524001.0002.

J. D. MacEwen, Ph.D. served as the Laboratory Director for the THRU of the University of California, Irvine and as co-principal investigator with T. T. Crocker, M.D., Department of Community and Environmental Medicine. Acknowledgement is made to D. E. Uddin, C. D. Flemming, and R. K. Blasingame for their significant contributions and assistance in the preparation of this report.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

A ONE-YEAR INHALATION TOXICITY STUDY OF OTTO FUEL II

INTRODUCTION

Otto Fuel II is used by the U. S. Navy as a liquid propellant in torpedoes and other weapon systems. The chief component (≈75%) of Otto Fuel II is 1,2-propylene glycol dinitrate (PGDN). The balance of Otto Fuel II is comprised of 2-nitrodiphenylamine (2%) added as a stabilizer and di-n-butyl sebacate (23%) added as a desensitizer.

The constituent of Otto Fuel II that presents the major health concern is the nitrate ester, PGDN. Nitrate esters are known to produce vasodilation, headaches, nasal congestion, dizziness, nausea, and methemoglobinemia.

Acute and subchronic experimental studies of PGDN using laboratory animals have been conducted. An acute oral LD_{50} of 250 mg/kg in male Sprague-Dawley rats has been reported by Andersen and Mehl (1973). Primary skin irritation tests in rabbits conducted by Jones et al. (1972) were negative, while applications of PGDN to rabbit eyes produced conjunctival erythema 5 minutes after application. PGDN is readily absorbed through the skin. Rabbits dosed repeatedly with 4 g/kg PGDN via dermal application were weak and cyanotic after the second application; 13 of 14 animals were dead after the fifth application.

Inhalation studies involving animals were also conducted by Jones et al. (1972). Male Sprague-Dawley rats were exposed to 65 mg/m^3 PGDN vapor, 7 hours/day, 5 days/week for a total of 30 days. No toxic effects were seen. Hemoglobin parameters were unchanged and histopathologic examination of tissue did not show any exposure related lesions. Continuous 90-day inhalation exposures of rats, guinea pigs, monkeys, and dogs were conducted at three PGDN concentrations: 67 mg/m^3, 108 mg/m^3, and 236 mg/m^3. Rate of body weight gain was unaffected by PGDN exposure. Decreases in hemoglobin (63%) and hematocrit (37%) were observed in dogs exposed to 236 mg/m^3 PGDN. Methemoglobin levels were elevated in all species with dogs and monkeys showing the most dramatic increase. Iron-positive deposits were present in the livers, spleens, and kidneys of dogs and monkeys exposed to 236 mg/m^3 PGDN. Fatty changes in the livers and kidneys were also noted in the animals exposed to PGDN vapor.

Subcutaneous injections of an LD_{50} dose (≈400 mg/kg) of PGDN in rats caused almost complete conversion of hemoglobin to methemoglobin suggesting that the destruction of the oxygen carrying

capacity of the blood was the cause of death (Clark and Litchfield, 1969). Following injection of PGDN in rats, a marked decrease in blood pressure occurred, with the maximum decrease in blood pressure coinciding with the maximum level of PGDN in the blood. Inorganic nitrate was found to be the major urinary metabolite with only very small amounts of inorganic nitrite and propylene glycol mononitrate (PGMN) excreted. Andersen and Smith (1973) have suggested that the metabolism of PGDN involves inorganic nitrite as a reactive intermediate which is eventually converted to inorganic nitrate. A similar type of process has been suggested for ethylene glycol dinitrate (EGDN) by Clark and Litchfield (1967).

In 1974, Stewart et al. reported a series of experimental inhalation exposures of short duration to humans with PGDN vapor. Headaches occurred in a majority of the individuals exposed to 1.4 mg/m^3 PGDN for 4 hours or more. Repetitive exposure to this concentration produced a tolerance to the headaches, an effect that has previously been described for other organic nitrate compounds (Goodman and Gilman, 1980). No alteration in blood clinical chemistry values occurred in humans after a single 8-hour exposure or a few repeated 8-hour exposures to 1.4 mg/m^3, PGDN vapor. Increasing the concentration of PGDN to 3.4 mg/m^3 increased the severity of the headaches. After 8 hours of exposure to this concentration, all three subjects had abnormal modified Romberg tests. One individual was unable to perform a heel-to-toe test with his eyes open. Blood clinical chemistry values in individuals exposed to 3.4 mg/m^3 PGDN were unchanged.

There is no direct evidence that PGDN is a tumorigenic agent; however, a recent publication indirectly suggests this possibility. The report describes a 2-year feeding study of trinitroglycerin (TNG) to rats, mice, and dogs (Dacre et al., 1979; Ellis et al., 1984). Cholangiofibrosis and hepatocellular carcinomas were found in the rats surviving 2 years of exposure to food containing 1.0% TNG. These same lesions were observed to a lesser degree, in the surviving rats fed 0.1% TNG. PGDN and TNG are closely related in structure, and it was of interest to determine if long-term inhalation exposure to Otto Fuel II would result in tumor formation.

Exposure levels for this study were based on measured PGDN concentrations. The low level (1.4 mg/m^3) represented the Threshold Limit Value (TLV) in effect prior to 1980. This level was subsequently reduced to 0.1 mg/m^3 in 1981 and then raised to 0.3 mg/m^3 in 1983. The higher exposure level used in the study, 240 mg/m^3, effectively duplicated the upper exposure level utilized in the previous 90-day study conducted by Jones et al. (1972).

METHODS

Test Agent

Otto Fuel II is a red-orange, free flowing liquid with a distinctive odor. The physical properties are listed in Table 1.

TABLE 1. PHYSICAL PROPERTIES OF OTTO FUEL II[a]

Density:	1.232 g/mL (25°C)
Vapor Pressure:	0.0877 mm Hg (25°C)
Freezing Point:	-27.7°C
Boiling Point:	Decomposes above 121°C
Flash Point (Cleveland Open Cup):	130°C
Viscosity:	4.4 cp (25°C)
Surface Tension:	34.45 dynes/cm
Water Saturation:	0.31% (25°C)
Heat Capacity:	0.445 BTU/lb
Maximum Air Saturation Conc. (Calculated):	781 mg/m^3
Solubilities:	
Insoluble:	Water, ethylene glycol, propylene glycol
Very Slight Soluble:	Heptane, petroleum ether
Very Soluble:	Alcohols, benzene, carbon tetrachloride, hexane, chloroform, toluene, dibutyl phthalate, trichloroethylene, acetone

[a] From NAVORD OP-3368 and NAVMED P-5112.

Ten 5-gallon containers of Otto Fuel II were supplied to the Toxic Hazards Research Unit by the Navy Medical Research Institute/Toxicology Detachment. The individual samples were analyzed by High Pressure Liquid Chromatography upon receipt to insure that a consistent sample was available for the entire study. This analysis indicated that the average material composition was 74.3 ± 1.2% PGDN, 1.9 ± 0.6% 2-nitrodiphenylamine and 23.8 ± 1.2% di-n-butyl sebacate. A trace contaminant, orthochloronitrobenzene (OCNB), associated with the manufacture of 2-nitrodiphenylamine, was also found in the Otto Fuel II supply.

Contaminant Generation and Monitoring

The great difference in contaminant concentrations between the two dome atmospheres, as well as safety considerations necessitated separate generation systems.

The contaminant introduction system for the 1.4 mg/m^3 PGDN exposure consisted of an agitated supply of Otto Fuel II maintained at a constant temperature (45°C). A controlled air sweep carried the necessary PGDN vapor (approximately 3 mg/min) to the chamber air input line. Chamber air flow was maintained at about 40 CFM. Concentration was controlled by a combination of Otto Fuel II temperature and chamber air flow rate.

Continuous concentration monitoring of the 1.4 mg/m^3 PGDN exposure was accomplished by a Miran IA infrared analyzer equipped with a 20 meter path length cell using the 12 micron band with a 2 mm slit.

The contaminant introduction system for the 240 mg/m^3 PGDN exposures consisted of three large electrically heated evaporator towers. Each tower had a contaminant flow rate of approximately 0.33 mL/min with a counter-current air flow of 5 CFM. The vapor passed through 1" stainless steel lines to the chamber input air line. Non-volatized material was pumped from the bottom of the towers to a container for disposal.

A Beckman 400 hydrocarbon analyzer modified to act as a loop injected isothermal (120°F) gas chromatograph was used to analyze the 240 mg/m^3 PGDN exposure chamber atmosphere. The automatic injection system provided a sample every 5 minutes. An 8 cm x 1/8" nickel column packed with 10% UCW-98 on Chromosorb W-HP separated PGDN with peak elution 2 minutes after injection.

Animals

CDF (Fischer 344)/CrlBr rats were obtained from Charles River Breeding Laboratories, Wilmington, Massachusettes. C57BL/6 mice were obtained from Jackson Laboratories, Bar Harbor, Maine. Purebred beagle dogs were purchased from Laboratory Research Enterprises, Kalamazoo, Michigan. Animals were gang caged by sex and species in stainless, wire mesh cages during exposure. Food (Purina, St. Louis, Missouri) was available during non-exposure periods. Water was available ad libitum. All cage areas were cleaned daily. Temperature and relative humidity were maintained at 22°C ± 2 and 50% ± 10, respectively.

Experimental Approach

Exposures were conducted in 25 m^3 Thomas Dome inhalation chambers on an industrial type work schedule of 6 hours/day, 5 days/week for a 1-year period. Exposures were not conducted on holidays or weekends. Dogs, rats, and mice were exposed to 1.4 mg/m^3 PGDN. Rats and mice were exposed to 240 mg/m^3 PGDN. A separate group of dogs, rats, and mice were held at the Veterinary Science Division Building, at Wright-Patterson Air Force Base, to serve as unexposed controls. The number of animals in each group is shown in Table 2. Because of space limitations within the chambers the inclusion of dogs at the 1.4 mg/m^3 exposure level necessitated a reduction in the numbers of rats and mice at that concentration.

TABLE 2. NUMBER OF ANIMALS IN EACH STUDY GROUP

PGDN Concentration	Dogs (Beagle)		Rats (Fischer 344)		Mice (C57BL/6)	
	M	F	M	F	M	F
0	3	3	100	100	100	100
1.4 mg/m^3	3	3	75	75	75	75
240 mg/m^3	-	-	100	100	100	100

At the completion of the 1-year exposure 10 male and 10 female rats and 10 male and 10 female mice from each experimental group were randomly chosen for necropsy and tissue collection. The remaining rodents were held for 1 year of postexposure observation. The exposure of dogs to 1.4 mg/m^3 PGDN was continued for an additional 60 days to investigate blood abnormalities. At the conclusion of this extra exposure time all of the exposed and control dogs were necropsied.

All animals that died or were sacrificed in this study were necropsied. The tissues listed in Table 3 were fixed for histopathologic examination. All of these tissues were examined for microscopic changes in the control and 240 mg/m^3 PGDN groups. However, the list of tissues examined from the rodents exposed to 1.4 mg/m^3 PGDN was reduced in accordance with current National Toxicology Program guidelines. In this group all lungs, livers, kidneys, and gross lesions were examined. In addition, all tissues showing evidence of a dose related effect (based on observation of the control and high level groups) were examined.

TABLE 3. TISSUES SAMPLED FROM ANIMALS EXPOSED TO OTTO FUEL II VAPOR

Gross lesions	Skin
Tissue masses or suspect tumors and regional lymph nodes	Mandibular lymph node
Larynx	Mammary gland
Trachea	Salivary gland
Lungs and bronchi	Stomach
Heart	Duodenum
Thyroids	Ileum
Parathyroids	Colon
Esophagus	Anus
Liver	Mesenteric lymph node
Sternebrae, vertebrae or femur (plus marrow)	Thigh muscle
Spleen	Sciatic nerve
Kidneys	Thymus
Bladder	Gallbladder
Nasal cavity	Pancreas
Brain	Seminal vesicles
Bone marrow smear	Prostate
	Testes
	Ovaries
	Uterus
	Pituitary

All animals were observed closely during the study to monitor signs of toxicity. Animals found in a moribund condition were sacrificed for tissue collection. Rats were individually weighed at biweekly intervals during exposure and monthly during the postexposure period. Dogs were individually weighed at biweekly intervals during the exposure. Mice were weighed in groups with the group mean weights followed on a monthly basis throughout the experimental period. Blood samples were drawn via the jugular vein from the dogs at biweekly intervals and clinical chemistry and hematology determinations made for the series of tests shown in Table 4. These same tests were performed on blood collected from the rats sacrificed at the conclusion of the exposure phase of the study and at 1-year postexposure. Blood from the rats was collected via the vena cava. Orbital sinus blood samples were also collected from rats at 6 months of exposure for hematology determinations.

TABLE 4. CLINICAL HEMATOLOGY AND CHEMISTRY TESTS PERFORMED ON DOGS AND RATS EXPOSED TO OTTO FUEL II VAPOR

Hematology	Chemistry
Hematocrit	Albumin
Hemoglobin	Total Protein
Total RBC	Glucose
Total WBC	Alkaline Phosphatase
Reticulocytes	SGPT
Differentials	SGOT
a. Bands	Bilirubin
b. Neutrophils	a. Total
c. Lymphocytes	b. Direct
d. Monocytes	Creatinine
e. Eosinophils	BUN
f. NRBC	γ-Glutamyl Transferase
Mean Corpuscular Volume (MCV)	
Mean Corpuscular Hemoglobin (MCH)	
Mean Corpuscular Hemoglobin Concentration (MCHC)	

Methemoglobin measurements were conducted according to the method of Rodkey, et al. (1979). Blood was collected from the tail vein of 10 male and 10 female rats in the control and high level exposure group. Dog blood was collected via the jugular vein. Liver, kidney, and spleen weights were measured in all dogs and rats at the exposure termination and postexposure termination sacrifices.

Statistical Analysis

A one-way analysis of variance technique was used for a statistical evaluation for equality of means. This was followed by a multiple comparison procedure when necessary (Zar, 1974). A probability of $p < 0.05$ was accepted as significant.

RESULTS

Analytical

The final exposure concentrations are shown in Table 5. Rats and mice received a total of 249 6-hour exposures during the year. Because of the extension of the exposure to dogs this species received 293 exposures.

TABLE 5. SUMMARY OF CHAMBER CONCENTRATION INFORMATION

Species	Target (mg/m^3)	Total Exposure Days	Analyzed Conc. (mg/m^3) Mean	SD
Rat, Mouse	1.4	249	1.43	0.08
Dog	1.4	293	1.43	0.08
Rat, Mouse	240	249	241	5.19

Exposure of Dogs to Otto Fuel II

There were no mortalities and dogs exposed to 1.4 mg/m^3 PGDN demonstrated no overt signs of toxicity during the exposure period. Biweekly body weight measurement did not reveal any significant differences between dogs exposed to 1.4 mg/m^3 PGDN and unexposed controls.

After 2 weeks of exposure, decreased hematocrit (Figure 1) and hemoglobin (Figure 2) values were evident in the dogs exposed to 1.4 mg/m^3 PGDN when compared to the unexposed control dogs. Red blood cell counts in exposed dogs were also depressed after 4 weeks of exposure (Figure 3). The anemia present in the Otto Fuel II exposed dogs produced no increase in reticulocyte counts; rather, a progressive decrease in reticulocytes appeared in the exposed dogs (Figure 4). Examination of dog RBC's after 6 and 8 months of exposure failed to reveal the presence of Heinz bodies.

During the last quarter of the exposure period, a substantial steady decrease in RBC counts, hematocrit, and hemoglobin values of exposed dogs occurred. These decreases were also noted at a somewhat earlier time in the control dogs. Because it was not clear at the end of the scheduled year-long exposure if the changes were persistent or transient, or if the changes were exposure related, the exposure of dogs was extended an additional 60 days. During this time period RBC, hematocrit, and hemoglobin values of both groups generally increased. However, in spite of this variation the values of dogs exposed to Otto Fuel II vapor continued to remain lower than controls during the time period. Despite the apparent anemia present in the exposed dogs, the reticulocyte counts did not increase. All other hematology and clinical chemistry measurements made on dog blood revealed no significant trends.

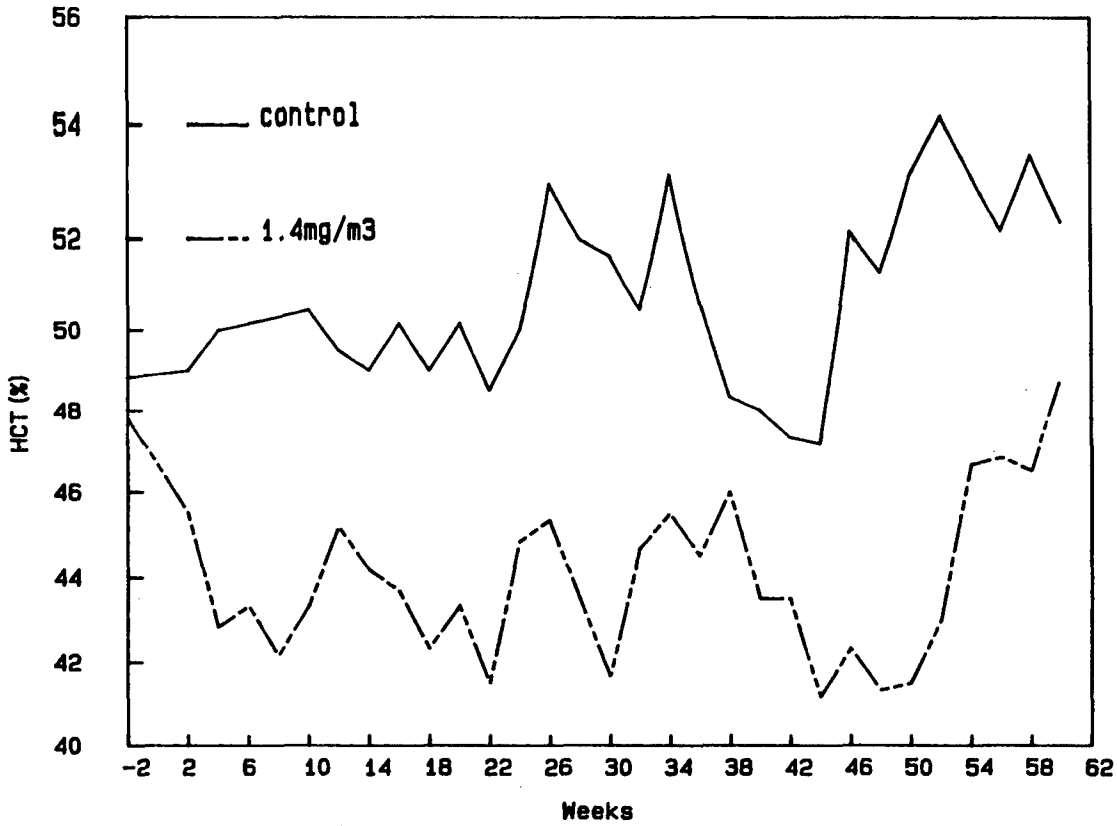

Figure 1. Effect of Otto Fuel II vapor exposure on dog hematocrit.

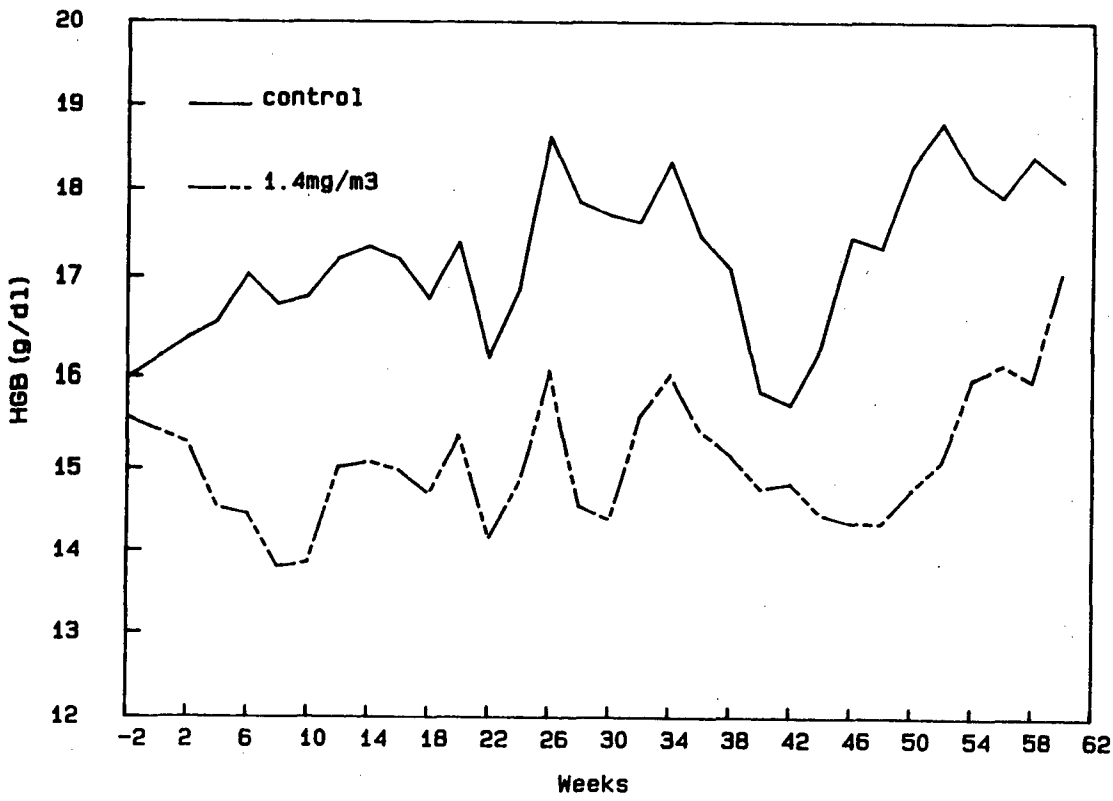

Figure 2. Effect of Otto Fuel II vapor exposure on dog hemoglobin.

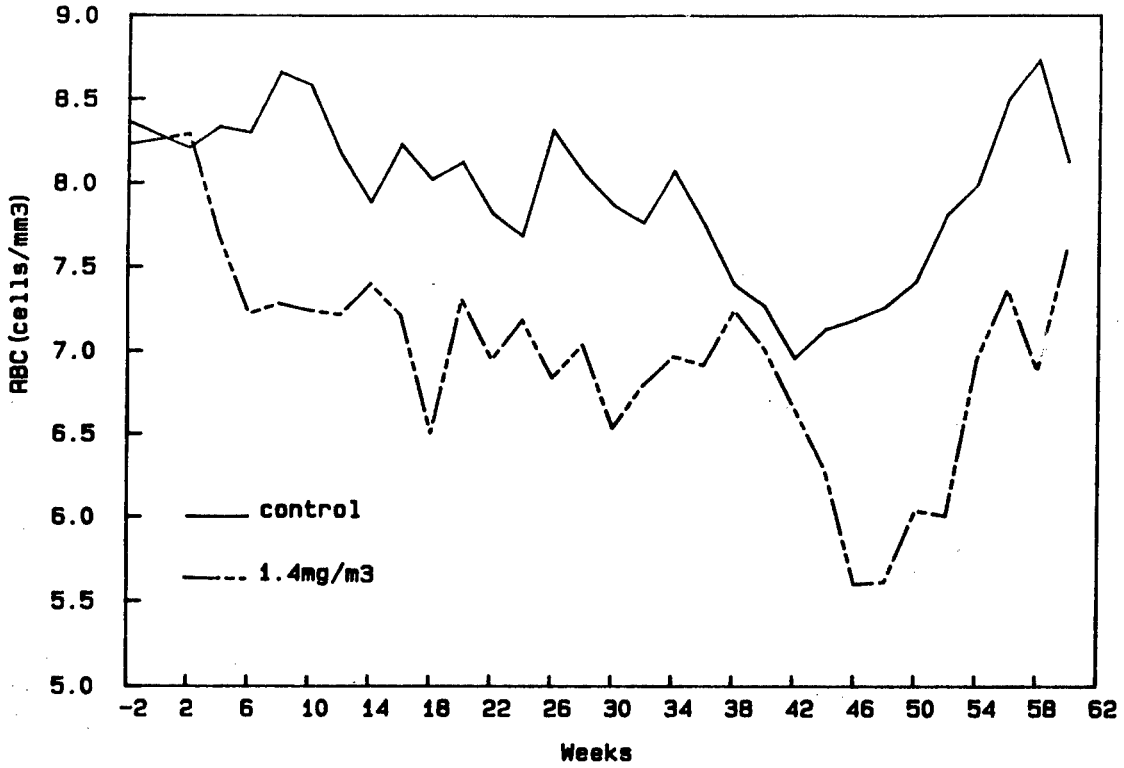

Figure 3. Effect of Otto Fuel II vapor exposure on dog red blood cell count.

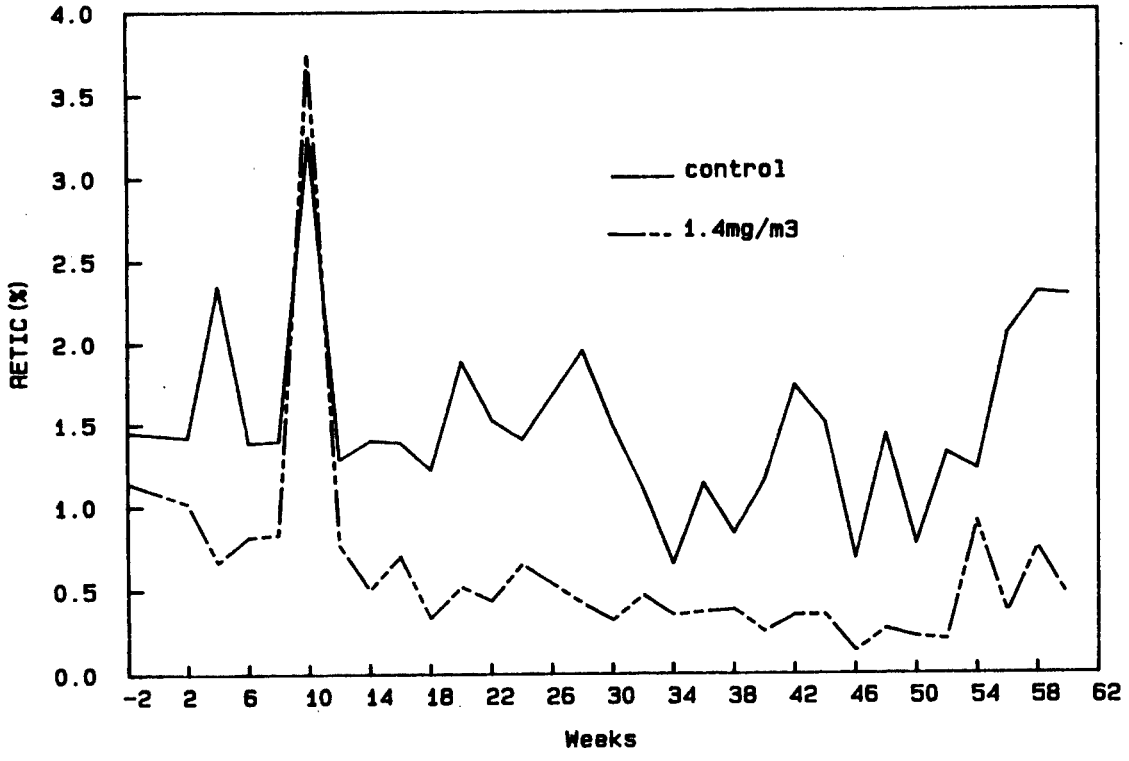

Figure 4. Effect of Otto Fuel II vapor exposure on dog reticulocyte counts.

Red blood cell osmotic fragility tests were conducted during the seventh month of the exposure to investigate the possibility that the anemia present in the exposed dogs was a result of increased fragility. The results of these tests are shown in Figure 5. The fragility curves for exposed and control animals were very similar. The only difference is that the blood from exposed dogs was more completely hemolyzed at lower saline concentrations than the controls. The test was repeated at 8 months exposure with similar results.

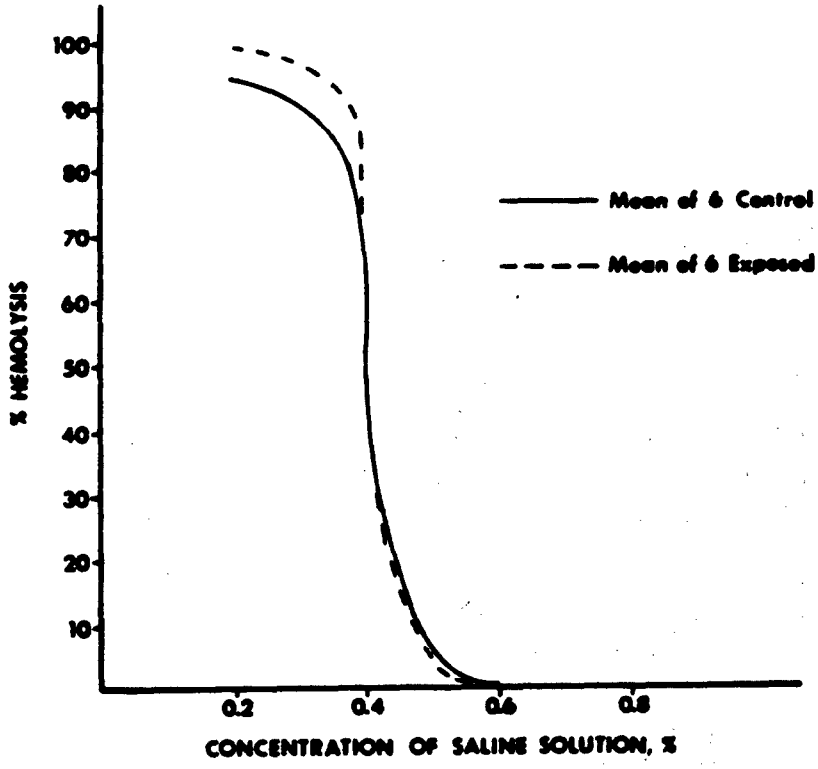

Figure 5. Osmotic fragility of dog blood after 7 months intermittent exposure to 1.4 mg/m^3 Otto Fuel II.

The results of methemoglobin determinations conducted on dogs are shown in Table 6. Exposure to 1.4 mg/m^3 PGDN produced a slight but statistically significant increase in methemoglobin.

Dog organ weights obtained at the time of sacrifice are shown in Table 7. A reduced liver/body weight ratio was noted in the dogs exposed to 1.4 mg/m^3 PGDN.

Microscopic examination of the tissues collected from dogs indicated no lesions that were considered to be exposure related. Examination of the bone marrow taken in routine bone sections did not suggest any unusual effects on the blood forming cells.

TABLE 6. METHEMOGLOBIN LEVELS (%)[a] IN DOGS EXPOSED TO OTTO FUEL II VAPOR

Exposure Day	PGDN Concentration (mg/m^3) 0	1.4
44	0.38 ± 0.15	1.21 ± 0.48[b]
136	1.79 ± 0.87	4.60 ± 1.51[b]
171	1.39 ± 0.82	2.84 ± 0.57[b]
231	1.37 ± 0.30	3.09 ± 0.95[b]

[a] Mean ± SD, N = 6.
[b] Different from control, $p < 0.05$.

TABLE 7. ORGAN WEIGHTS[a] OF DOGS EXPOSED TO OTTO FUEL II VAPOR

	PGDN Cncentration (mg/m^3) 0	1.4
Body weight, kg	11.7 ± 2.9	13.2 ± 0.9
Liver weight, g	336.9 ± 76.7	318.8 ± 30.9
Liver/100 g body wt	2.9 ± 0.2	2.4 ± 0.3[b]
Spleen weight, g	117.9 ± 19.1	163.4 ± 53.3
Spleen/100 g body wt	1.1 ± 0.3	1.3 ± 0.4
Kidney weight, g	56.6 ± 15.8	62.0 ± 6.7
Kidney/100 g body wt	0.5 ± 0.1	0.5 ± 0.1

[a] Mean ± SD, N = 6.
[b] Different from control, $p < 0.05$.

Exposure of Mice to Otto Fuel II

Exposure to 1.4 mg/m^3 or 240 mg/m^3 PGDN did not alter the body weight gain of male or female mice. Statistical analysis (Mantel-Cox test) of mouse survival data indicated a slight but significant ($p < 0.01$) increase in the life span of exposed male mice when compared to controls. This increased life span was not seen in female mice.

Table 8 presents the results of the microscopic examination of tissues collected from mice exposed to Otto Fuel II. Only the most frequently observed lesions are listed. At exposure termination, chronic ulcerative dermatitis was recorded in 11/39 (28%) of male and female control mice and only 2/41 (5%) of the high dose subjects. Hyaline degeneration of the nasal epithelium and granulocytic hyperplasia of the bone marrow were recorded more frequently in control mice than in the exposed subjects. Hyaline degeneration of gallbladder epithelium was also noted frequently in control mice. Hepatocellular fatty change was noted with increased frequency in control mice. This change was often associated with chronic ulcerative dermatitis. In contrast, hepatocellular cytoplasmic vacuolization was recorded exclusively in exposed mice where 12/35 (34%) of the high dose mice exhibited this change. At the light microscopic level, cytoplasmic vacuolization was attributed to increased cytoplasmic glycogen and probably was related to an abbreviated routine fasting period just prior to sacrifice and not to any pathologic event.

Lesions observed at the conclusion of the 1-year postexposure observation period were generally consistent with those observed at exposure termination (Table 8). In addition, extramedullary hematopoiesis in the spleen was observed more frequently in controls. Splenic lymphoid hyperplasia was noted to be slightly more common in mice exposed to 240 mg/m^3 PGDN than in controls. Both atrophy and focal hyperplasia were common in the adrenal cortex of older male mice assigned to all experimental groups. All other tissue changes noted in mice examined at either exposure termination or 1-year postexposure demonstrated no relationship to exposure.

Exposure of Rats to Otto Fuel II

Similar to the effect noted in male mice, the female rats exposed to Otto Fuel II demonstrated a significantly ($p < 0.01$) longer life span compared to controls. The survival of male rats exposed to Otto Fuel II was comparable to controls.

Exposure to Otto Fuel II retarded the growth of male rats compared to unexposed controls (Figure 6). This effect appeared to be dose related, however, the magnitude of the exposure concentration difference was not reflected by the difference in body weights between the two exposure groups.

TABLE 8. LESIONS[a] OBSERVED IN MICE EXPOSED TO OTTO FUEL II VAPOR

	Male			Female		
	Control	1.4 mg/m^3	240 mg/m^3	Control	1.4 mg/m^3	240 mg/m^3
	Exposure Termination					
Skin						
Ulcer	3/21 (14)	2/16 (13)	2/17 (12)	8/18 (44)	0/18 (0)[b]	0/24 (0)[b]
Nasal						
Hyaline degeneration	11/21 (52)	7/16 (44)	1/14 (7)[b]	13/17 (76)	8/15 (53)	4/18 (22)[b]
Bone Marrow						
Granulocytic hyperplasia	13/20 (65)	3/13 (23)[c]	1/14 (7)[b]	12/16 (67)	0/14 (0)[b]	0/18 (0)[b]
Liver						
Fatty change	12/21 (57)	2/15 (13)[c]	5/15 (33)	10/17 (59)	9/16 (56)	3/20 (15)[b]
Vacuolization	0/21 (0)	3/15 (20)	3/15 (20)	0/17 (0)	3/16 (19)	9/20 (38)[b]
Gall Bladder						
Hyaline degeneration	6/19 (29)	1/12 (8)	0/11 (0)	3/16 (19)	3/12 (25)	1/14 (7)
	1-Year Postexposure					
Skin						
Ulcer	5/79 (6)	3/59 (5)	5/83 (6)	17/82 (21)	9/57 (16)	11/75 (15)
Mammary Gland						
Cystic hyperplasia	0/79 (0)	d	0/75 (0)	28/82 (34)	d	4/75 (5)[b]
Bone						
Osteosarcoma	0/70 (0)	d	0/82 (0)	0/76 (0)	d	1/70 (1)
Osteosclerosis	0/70 (0)	d	1/82 (1)	0/76 (0)	d	0/70 (0)
Bone Marrow						
Granulocytic hyperplasia	30/70 (43)	d	6/81 (7)[b]	26/74 (35)	d	12/69 (17)[c]
Liver						
Fatty change	39/79 (49)	28/57 (49)	54/82 (66)	52/81 (64)	19/56 (34)	39/75 (52)
Spleen						
Hematopoiesis	24/76 (32)	4/14 (29)	16/79 (20)	49/78 (63)	15/35 (43)	23/73 (32)[b]
Lymphoid hyperplasia	2/76 (3)	2/14 (14)	15/79 (19)[b]	2/78 (2)	3/35 (9)	7/73 (10)
Adrenal Gland						
Atrophy	12/74 (16)	36/42 (86)[b]	15/72 (21)	0/80 (0)	0/9 (0)	1/72 (1)
Salivary Gland						
Lymphocytic infiltrates	45/73 (62)	d	47/81 (57)	38/74 (51)	d	35/72 (49)

[a] Number observed/Number examined (%).
[b] Different from control, $p < 0.01$.
[c] Different from control, $p < 0.05$.
[d] Tissues not examined.

The body weights of female rats are shown in Figure 7. During the exposure period the body weights of Otto Fuel II exposed rats were comparable to or greater than controls. Subsequent weighings postexposure indicated that both groups of exposed rats weighed less than controls. As with the male rats, the small differences in body weights between the exposure groups did not reflect the large difference in exposure concentration.

Examination of male and female rat clinical chemistry parameters at exposure termination and 1-year postexposure did not indicate any clearly exposure related effects. For comparison with the dog erythrocyte parameters, red blood cell (RBC) counts,

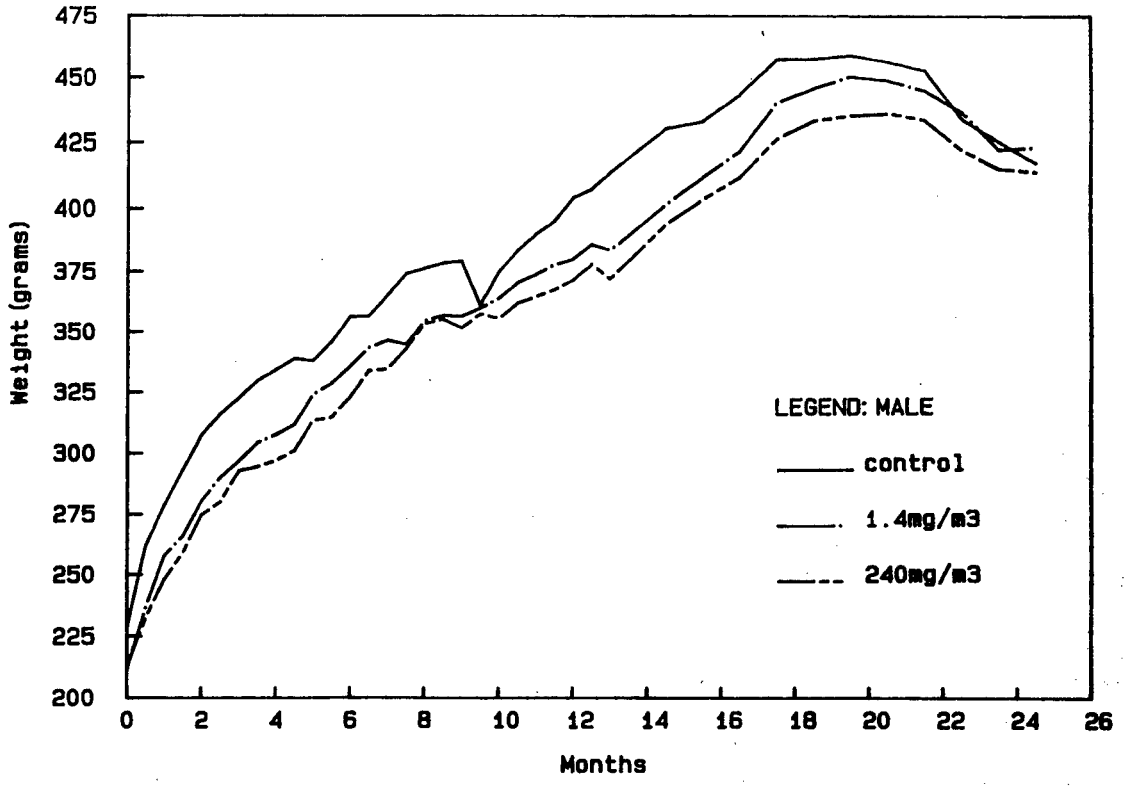

Figure 6. Effects of Otto Fuel II vapor exposure on male rat body weight.

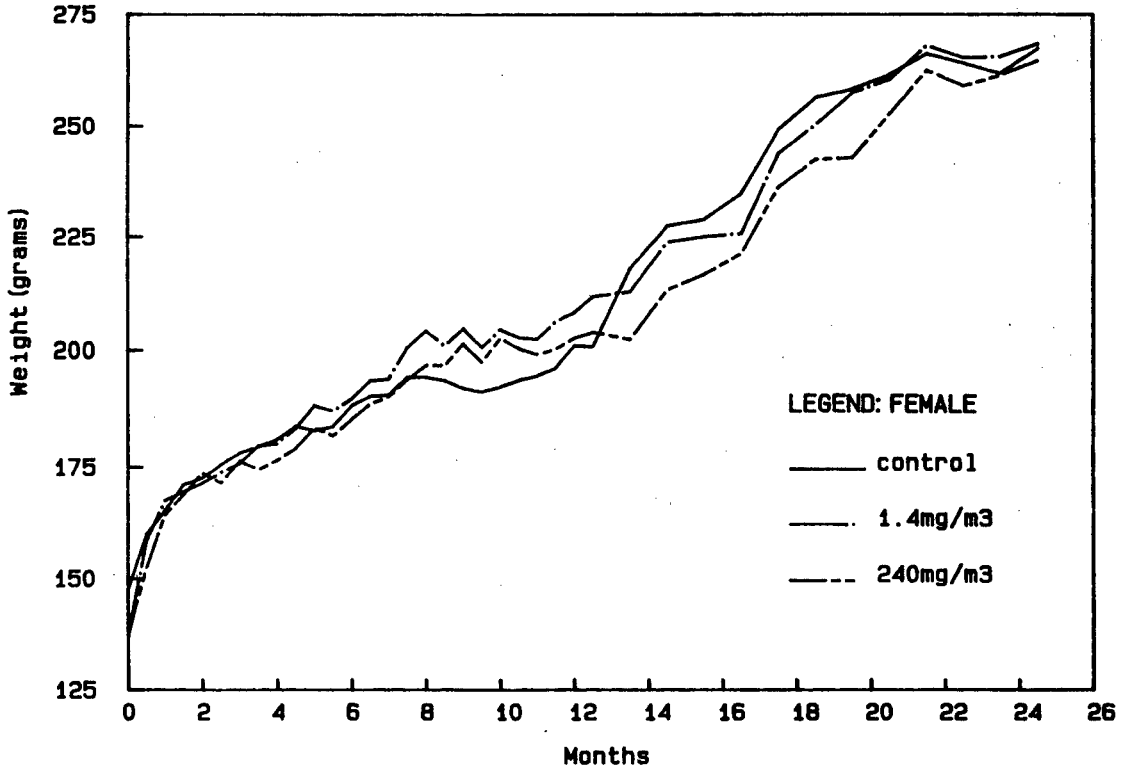

Figure 7. Effects of Otto Fuel II vapor exposure on female rat body weight.

hematocrit (HCT), and hemoglobin (HGB) levels are presented in Table 9. Although there were occasional differences in erythrocyte parameters between control and exposed rats, there was generally no indication of a consistent dose response. Furthermore, none of the groups examined demonstrated reductions in all three parameters (RBC, HCT, and HGB) at any single examination time.

TABLE 9. RED BLOOD CELL COUNTS (RBC) HEMATOCRIT (HCT) AND HEMOGLOBIN (HGB) VALUES[a] OF MALE AND FEMALE RATS EXPOSED TO OTTO FUEL II VAPOR

	Male Rats			Female Rats		
	PGDN Concentration (mg/m^3)			PGDN Concentration (mg/m^3)		
	0	1.4	240	0	1.4	240
6-Month Exposure						
RBC ($x10^6$ cells/mm^3)	8.31 ± 0.47	8.22 ± 0.49	7.50 ± 0.77[b]	9.31 ± 1.29	8.48 ± 0.97	7.79 ± 2.01[c]
HCT (%)	50 ± 2	49 ± 2	50 ± 1	48 ± 2	50 ± 2[c]	48 ± 1
HGB (g/dl)	17.3 ± 0.4	16.8 ± 0.7[c]	17.2 ± 0.4	16.6 ± 0.9	17.4 ± 0.6[c]	16.9 ± 0.3
1-Year Exposure						
RBC ($x10^6$ cells/mm^3)	9.65 ± 1.10	7.53 ± 0.43[b]	8.83 ± 0.68[c]	6.33 ± 0.96	7.55 ± 0.99[b]	7.44 ± 0.71[c]
HCT (%)	46 ± 2	44 ± 2[b]	47 ± 1	41 ± 1	44 ± 1[c]	44 ± 1
HGB (g/dl)	14.8 ± 0.4	14.6 ± 0.7	15.0 ± 0.3	13.8 ± 1.4	14.7 ± 0.3	14.7 ± 0.3
1-Year Postexposure						
RBC ($x10^6$ cell/mm^3)	8.51 ± 1.30	8.60 ± 1.48	8.36 ± 1.00	7.14 ± 0.69	7.39 ± 0.68	7.30 ± 0.42
HCT (%)	45 ± 6	44 ± 7	44 ± 4	39 ± 4	41 ± 4	40 ± 2
HGB (g/dl)	17.2 ± 2.0	17.3 ± 2.6	16.8 ± 2.0	14.5 ± 1.7	15 ± 1.0	14.8 ± 0.8

[a] Mean ± SD, N = 9 - 11 samples/group.
[b] Different from control, $p < 0.01$.
[c] Different from control, $p < 0.05$.

Results of the rat methemoglobin tests conducted during the exposure period are shown in Table 10. Exposure to 240 mg/m^3 produced a mild increase in methemoglobin formation in both male and female rats.

Rat organ weights are shown in Table 11. Organ weights relative to body weight are shown in Table 12. At exposure termination, male and female rats exposed to Otto Fuel II demonstrated reduced liver weight. Considering the large concentration difference between the two exposure groups, the small difference in liver weights would suggest this finding was unrelated to exposure. Other organ weight changes noted were also considered incidental.

Lesions observed in rats at exposure termination are shown in Table 13. The table has been abbreviated to exclude most of the common spontaneous lesions showing no exposure relationship. At exposure termination, a modest number of rats assigned to both the low and high dose exposure groups exhibited mild hyaline degeneration of the nasal epithelium. Very slight increases in

TABLE 10. METHEMOGLOBIN LEVELS[a] (%) IN RATS EXPOSED TO OTTO FUEL II VAPOR

Exposure Day	Sex	PGDN Concentration (mg/m^3) 0	240
3	M	0.97 ± 0.92	1.84 ± 1.10
8	F	0.81 ± 0.70	2.00 ± 1.70
13	M	0.40 ± 0.31	1.59[b] ± 0.68
18	F	0.71 ± 0.51	1.58[b] ± 0.64
23	M	0.62 ± 0.35	2.90[b] ± 0.69
28	F	1.19 ± 0.71	2.31[b] ± 0.95
33	M	1.20 ± 0.71	2.49[b] ± 1.33
145	M	1.48 ± 0.82	3.57[b] ± 0.52
170	F	2.51 ± 0.52	3.18[b] ± 1.05
212	M	2.22 ± 0.93	3.59[b] ± 1.47
241	F	1.39 ± 0.42	3.08[b] ± 1.33
245	M	1.30 ± 0.96	3.76[b] ± 0.67

[a] Mean ± SD, N = 7-10 samples/group.
[b] Different from control, $p < 0.05$.

TABLE 11. ORGAN WEIGHTS[a] OF RATS EXPOSED TO OTTO FUEL II VAPOR

Time	PGDN Conc. (mg/m^3)	Weight (g) Body	Liver	Kidney	Spleen
		Male Rats			
Exposure Termination	0	407 ± 23	10.54 ± 0.93	2.43 ± 0.17	0.61 ± 0.09
	1.4	379 ± 29[b]	9.47 ± 0.93[b]	2.30 ± 0.18	0.55 ± 0.07
	240	378 ± 17[b]	9.40 ± 0.87[c]	2.33 ± 0.13	0.57 ± 0.08
1-Year Postexposure	0	391 ± 24	11.52 ± 0.84	3.00 ± 0.36	0.96 ± 0.21
	1.4	424 ± 38[b]	11.38 ± 1.58	2.90 ± 0.21	1.18 ± 0.27
	240	394 ± 19	11.14 ± 1.72	2.79 ± 0.21	1.08 ± 0.52
		Female Rats			
Exposure Termination	0	216 ± 29	5.93 ± 1.09	1.50 ± 0.17	0.43 ± 0.11
	1.4	206 ± 13	4.89 ± 0.33[b]	1.39 ± 0.06	0.38 ± 0.05
	240	200 ± 14	4.71 ± 0.32[c]	1.43 ± 0.08	0.35 ± 0.06[b]
1-Year Postexposure	0	254 ± 17	6.94 ± 0.67	1.79 ± 0.14	0.93 ± 0.48
	1.4	249 ± 41	6.90 ± 0.94	1.79 ± 0.15	0.62 ± 0.27
	240	252 ± 21	6.62 ± 0.67	1.74 ± 0.14	0.55 ± 0.26[b]

[a] Mean ± SD, N = 9 - 10 samples/group.
[b] Different from control, $p < 0.05$.
[c] Different from control, $p < 0.01$.

TABLE 12. RELATIVE ORGAN WEIGHTS[a] OF RATS EXPOSED TO OTTO FUEL II VAPOR

Time	PGDN Conc. (mg/m^3)	Relative Weight		
		Liver	Kidney	Spleen
		Male Rats		
Exposure	0	2.59 ± 0.17	0.60 ± 0.04	0.15 ± 0.02
Termination	1.4	2.49 ± 0.10	0.61 ± 0.02	0.14 ± 0.01
	240	2.48 ± 0.16	0.62 ± 0.03	0.15 ± 0.02
1-Year	0	2.95 ± 0.17	0.77 ± 0.10	0.25 ± 0.05
Postexposure	1.4	2.69 ± 0.30	0.69 ± 0.04[c]	0.28 ± 0.07
	240	2.83 ± 0.40	0.71 ± 0.04	0.27 ± 0.13
		Female Rats		
Exposure	0	2.74 ± 0.26	0.69 ± 0.03	0.20 ± 0.03
Termination	1.4	2.38 ± 0.11[b]	0.68 ± 0.03	0.18 ± 0.03
	240	2.36 ± 0.18[b]	0.72 ± 0.04	0.17 ± 0.02
1-Year	0	2.74 ± 0.22	0.71 ± 0.03	0.37 ± 0.21
Postexposure	1.4	2.79 ± 0.20	0.74 ± 0.14	0.25 ± 0.08
	240	2.64 ± 0.28	0.69 ± 0.04	0.22 ± 0.12

[a] Organ weight/100 g body weight, Mean ± SD, N = 9-10 samples/group.
[b] Different from control, $p < 0.01$.
[c] Different from control, $p < 0.05$.

TABLE 13. LESIONS[a] OBSERVED IN RATS EXPOSED TO OTTO FUEL II VAPOR

	Male			Female		
	Control	1.4 mg/m^3	240 mg/m^3	Control	1.4 mg/m^3	240 mg/m^3
	Exposure Termination					
Nasal						
Hyaline degeneration	0/15 (0)	5/15 (30)[b]	5/11 (45)[c]	0/17 (0)	3/10 (30)	2/13 (15)
Lung						
Perivascular cuffing	1/15 (7)	4/15 (27)	1/11 (9)	1/17 (6)	1/10 (10)	6/13 (46)[b]
Alveolar macrophages	0/15 (0)	1/15 (7)	1/11 (9)	0/17 (0)	0/10 (0)	0/13 (0)
Congestion	3/15 (30)	3/15 (2)	0/11 (0)	3/17 (18)	0/10 (0)	2/13 (15)
Lymphocytic infiltration	0/15 (0)	2/15 (13)	0/11 (0)	0/17 (0)	0/10 (0)	0/13 (0)
	1-Year Postexposure					
Nasal						
Hyaline degeneration	18/82 (22)	d	23/87 (26)	51/83 (61)	d	59/85 (69)
Bone						
Osteosclerosis	0/78 (0)	0/55 (0)	0/85 (0)	10/80 (13)	5/61 (8)	24/83 (29)[b]
Osteosarcoma	0/78 (0)	1/55 (2)	2/85 (2)	0/80 (0)	0/61 (0)	0/83 (0)
Osteoma	0/78 (0)	0/55 (0)	0/85 (0)	0/80 (0)	1/61 (2)	0/83 (0)
Spleen						
Mononuclear cell leukemia	12/82 (15)	6/58 (10)	5/88 (6)	22/82 (27)	19/65 (29)	6/87 (7)[c]
Hemosiderosis	7/82 (9)	1/58 (2)	8/88 (9)	34/82 (41)	5/65 (8)[c]	53/87 (61)[b]
Uterus						
Adenocarcinoma	-	-	-	24/81 (30)	3/65 (5)[c]	6/85 (7)[c]
Endometrial stromal polyp	-	-	-	3/81 (4)	7/65 (11)	12/85 (14)

[a] Number observed/Number examined (%).
[b] Different from control, $p < 0.05$.
[c] Different from control, $p < 0.01$.
[d] Tissues not examined.

pulmonary inflammatory changes (including perivascular cuffing, lymphocytic infiltrates, chronic/interstitial inflammation, and alveolar macrophages) were noted in exposed subjects.

Changes noted in rats at the conclusion of 1-year postexposure are also shown in Table 13. Primary bone neoplasms (1 osteoma and 3 osteosarcomas) were observed in exposed rats whereas no skeletal tumors were recorded in control subjects. Osteosclerosis of cortical bone was also noted with modest frequency in the high dose females. Mononuclear cell leukemia (large granular lymphocyte leukemia) was observed with diminished frequency in both male and female exposed rats. Splenic hemosiderosis was frequently diagnosed in both control and high dose females. Uterine (endometrial) adenocarcinomas were recorded in 30% of the control females whereas only 7% of the high dose females exhibited this neoplasm. Tumor incidences in both groups appeared to be within the range of normal variability for uterine adenocarcinomas in Fischer 344 rats. In contrast to the decreasing trend for uterine adenocarcinomas in exposed rats, endometrial stromal polyps were slightly increased in exposed females, 14% in high dose subjects compared with 11% in low dose females and 4% in controls. Endometrial stromal polyps are common in older Fischer 344 rats. All other changes noted in male and female rats from the study were considered to be unrelated to Otto Fuel II exposure.

DISCUSSION

Of the three species of animals exposed to Otto Fuel II vapors in this study the dog appeared to be the most sensitive. Previous 90-day continuous inhalation studies by Jones et al. (1972) indicated that dogs exposed to 236 mg/m^3 PGDN showed markedly reduced hemoglobin and hematocrit levels. In addition these dogs developed increased methemoglobin levels. The results of the present study in which dogs were exposed for a longer time period at a much lower concentration (1.4 mg/m^3) indicate a similar, albeit, less severe response. Reductions in RBC counts, hematocrit, and hemoglobin levels were evident in exposed dogs within the first month of exposure, and continued to be depressed for the entire period of exposure. Subsequent examinations conducted during the course of the exposure indicated continued significant differences in these erythrocyte parameters between exposed and control dogs. Because of the depression in these parameters noted near the original 1-year exposure termination date, the exposure was extended for 60 days. During this time the erythrocyte parameters increased, suggesting that the flucuations were within normal species variations. Despite these

variations in both control and exposed dogs, the relative difference between the two groups of animals remained and it was concluded that the differences were due to Otto Fuel II exposure. Concommitant with the reduced erythrocyte parameters in dogs was the presence of increased methemoglobin levels. Although these methemoglobin concentration increases were small, they were a consistent effect and were found to be statistically significant when compared to control values. Furthermore, comparison of the dog blood effects noted in this study with those of the previous study by Jones et al. (1972) demonstrates a dose response relationship.

Species differences in the formation of methemoglobin by nitrates have been reported by Calabrese et al. (1983). Jones et al. (1972) found dogs and monkeys to be more susceptible to PGDN-induced methemoglobin formation than rats or guinea pigs. Recent in vitro studies by Wyman et al. (1985) indicated the net rate of PGDN-mediated methemoglobin formation followed the sequence dog > guinea pig > rat > human. A similar relationship between the dog and rat was found in the present study. The methemoglobin concentration increases in rats exposed to PGDN for 1 year were generally comparable to the levels determined in dogs. When one considers the fact that these rats were exposed to PGDN at a concentration approximately 170 times greater than the dogs, the species difference in susceptability is readily apparent. In addition, rats failed to demonstrate the reductions in erythrocyte parameters that were consistently seen in dogs.

The presence of a trace contaminant, ortho-chloronitrobenzene (OCNB) was detected in the neat Otto Fuel II used for these exposures, in the distillate of volatilized Otto Fuel II, and in a vapor trap located in the introduction line of the low level exposure chamber. OCNB had been shown to produce late life tumors in male rats and hepatocellular tumors in female rats after sustained 12-month doses of 500 mg/kg of body weight (Weisburger et al., 1978). Calculations based on maximum OCNB found in distillate condensate, using average rat ventilation rates and assuming 100% retention, produced an estimated life dose for each rat of 0.05 mg/kg (Einhaus et al., 1982), an insignificant amount compared to the 500 mg/kg dose found to be carcinogenic.

The development of liver tumors in rats fed TNG for 2 years (Dacre et al., 1979; Ellis et al., 1984) suggested the possibility that long-term exposure to PGDN may also result in increased tumor formation, given the closely related structure of TNG and PGDN. The results of the microscopic examination of tissues collected from dogs, mice, and rats at exposure termination and at 1-year postexposure generally failed to suggest any exposure

related non-neoplastic changes. The only microscopically observable tissue alterations occurring more frequently in mice exposed to 240 mg/m^3 Otto Fuel II than in controls was splenic lymphoid hyperplasia. This finding may have represented early lymphoma, which is very common in older C57BL/6 mice. This increased incidence was considered incidental. Similarily, the non-neoplastic changes, including splenic hemosiderosis, noted in rats exposed to Otto Fuel II were also considered to be incidental effects unrelated to exposure. The incidence of osteosclerosis was thought to be related to variations in estrogenic hormone levels. In addition, no strong tumorigenic potential was demonstrated in rats or mice exposed to Otto Fuel II vapor, even at a high concentration approaching 800 times the present TLV of 0.3 mg/m^3. Interestingly, the frequency of mononuclear cell leukemia, a hematopoietic neoplasm usually very common in aging Fischer 344 rats, was noted with decreased frequency in exposed subjects, suggesting a possible protective effect. The occurrence of primary bone tumors in both the low dose and high dose rat groups, with none in the controls suggests a possible relationship with Otto Fuel II exposures, since these tumors are generally considered to be uncommon. However, the low frequency and absence of a dose response relationship, in view of the drastic difference in exposure concentration, implies that the development of these neoplasms was probably unrelated to Otto Fuel II exposure. More importantly, this study has demonstrated that despite the similarity in structure between TGN and PGDN, 1 year of intermittent inhalation exposure to PGDN produced no significant liver tumors in rats or mice.

REFERENCES

Andersen, M. E. and R. G. Mehl (1973), A comparison of the toxicology of triethylene glycol dinitrate and propylene glycol dinitrate, American Industrial Hygiene Association Journal 34, 12:526-532.

Andersen, M. E. and R. A. Smith (1973), On the mechanism of the oxidation of human and rat hemoglobin by propylene glycol dinitrate, Biochemical Pharmacology 22, 3247-3256.

Calabrese, E. J., G. S. Moore, and M. S. McCarthy (1983), The effect of ascorbic acid on nitrite-induced methemoglobin formation in rats, sheep, and normal human erythrocytes, Reg. Toxicol. Pharmacol. 3, 184-188.

Clark, D. G. and M. H. Litchfield (1967), Metabolism of ethylene glycol dinitrate and its influence on blood pressure of the rat, Brit. J. Ind. Med. 24, 320-325.

Clark, D. G. and M. H. Litchfield, (1969), The toxicity, metabolism, and pharmacologic properties of propylene glycol 1, 2-dinitrate, Toxicology and Applied Pharmacology 15, 175-184.

Dacre, J. D., C. Lee, H. V. Ellis, and C. Hong (1979), Chronic and carcinogenic study of trinitroglycerin in rats, mice, and dogs, Presented at the 21st Meeting of the European Society of Toxicology, Dresden, GDR.

Einhaus, R. L., J. D. MacEwen, E. H. Vernot, A. K. Roychowdhury, and R. L. Hilderbrand (1982), Analysis of orthochloronitrobenzene in Otto Fuel II by high pressure liquid chromatography, AFAMRL-TR-82-15, Air Force Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio.

Ellis, H. V., C. B. Hong, C. C. Lee, J. C. Dacre, and J. P. Llennon (1984), Subacute and chronic toxicity studies of trinitroglycerin in dogs, rats, and mice, Toxicol. Appl. Pharmacol., 4:248-260.

Goodman and Gilman (1980),In: The Pharmacological Basis of Therapeutics 6th Edition, (Gilman, A. G., Goodman, L. S., Gilman, A., Mayer, S. E., and Melmon, K. L., eds.), Mac Millan Publishing Company Inc., New York, p. 824.

Jones, R. A., J. A. Strickland, and J. Siegel (1972), Toxicity of propylene glycol 1, 2-dinitrate in experimental animals, Toxicology and Applied Pharmacology 22, 128-137.

Rodkey, F. L., T. A. Hill, L. L. Pitts, and R. F. Robertson (1979), Spectrophotometric measurement of carboxyhemoglobin and methemoglobin in blood, Clin. Chem., 25/8, 1388-1395.

Stewart, R. D., J. E. Peterson, P. E. Newton, C. L. Hoke, M. J. Hosko, A. J. Lebrun, and G. M. Lawton (1974), Experimental human exposure to propylene glycol dinitrate, Toxicology and Applied Pharmacology 30, 377-395.

Weisburger, E. K., A. B. Russfield, F. Homburger, J. H. Weisburger, E. Boger, C. G. VanDongen, and K. C. Chu (1978), Testing of twenty-one environmental aromatic amines or derivatives for long-term toxicity or carcinogenicity, Journal of Environmental Pathology and Toxicology, 2:235-356.

Wyman, J. F., B. H. Gray, L. H. Lee, J. Coleman, C. Flemming, and D. E. Uddin (1985), Interspecies variability in propylene glycol dinitrate-induced methemoglobin formation, Toxicol. Appl. Pharmacol., Vol. 81.

Zar, J. H. (1974), In: Biostatistical Analysis, Prentice-Hall, Englewood Cliffs, New Jersey.

☆ U.S. GOVERNMENT PRINTING OFFICE: 1986 - 646-006/20233